The adequacy of aging techniques in vertebrates for rapid estimation of population mortality rates from age distributions

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Appendix S3. Background information on seven different ageing techniques.

Eight aging techniques were reviewed because they are well known indicators that to some extent correlate with age (i.e. telomere length, otolith ring count, otolithometry, age-length keys and skeletochronology), or are promising indicators in determining age with low errors (i.e. DNA mythelation, sjTREC and racemization).

Telomere length

Telomeres are highly conservative DNA tandems at the ends of eukaryotic linear chromosomes. Considering the general principle of telomere shortening with aging, telomere length has mainly been employed as a biomarker for biological age in humans and other mammals, birds and fishes and less often in other animal taxa. Its correlation with age was reviewed in Dunshea et al. (2011). Besides age, telomere length also varies with an individual's health status and can for instance be used as an indicator of oxidative stress (Haussmann et al., 2005). Thus, telomere shortening may provide important information on mortality risk at the level of the individual (Heidinger et al., 2012). Obviously, the fact that telomere length and the rate of telomere shortening varies with other factors but age decreases its reliability for age determination.

For the measurement of telomere length three different methods have been employed in the literature, potentially leading to biases in the reported values. However, as 39 of the total of 40 studies used telomere restriction fragment length analysis (TRF) analysis, or validated their methods using TRF, which has been described as the original and standard method (Montpetit et al., 2014, Nussey et al., 2014) and only one study used qPCR

without TRF validation, we did not distinguish for these different methodologies when assessing the performance of telomere length.

DNA methylation

DNA methylation is an epigenetic modification, adding a methyl group to the cytosine or adenine residual. DNA methylation often occurs at the C5 position of cytosine residuals that are adjacent to guanidine residuals (CpG site) (Jones, 2012). DNA methylation increases or decreases with age, depending on the position of a specific CpG site (Hannum et al., 2013). Besides age, DNA methylation levels are affected by environmental factors, e.g. sun exposure (Gronniger et al., 2010), life style, e.g. consumption of alcohol and tobacco (Weidner et al., 2014) and diseases, e.g. diabetes (Gu et al., 2013). To improve the accuracy in age determination, DNA methylation levels are often examined in more than one CpG site within one or more genes. Its application to determine age has so far been tried in humans and other mammals (e.g. Polanowski et al., 2014, Zbiec-Piekarska et al., 2015). A more detailed review on this technique and its application can be found in Jarman et al. (2015).

sjTREC

The thymus rearranges T cell receptors to generate diverse T cells. During this rearrangement, small circles of DNA, signal-joint T cell receptor excision circles (sjTRECs), are being produced (Kong et al., 1998). Carrying sjTRECs, these T cells enter peripheral blood as circulating T cells. Circulating T cells replicate in peripheral blood, but sjTREC does not. sjTREC is consequently diluted with each cell division in peripheral blood (Takeshita et al., 1989). In addition, the ability of the thymus to generate T cells declines with age (Douek et al., 1998), meaning fewer sjTRECs are produced. As a result of the dilution effect in

45 combination with a reduced input from the thymus, sjTREC levels in peripheral blood
46 decline with age. Besides age, sjTREC levels can be reduced by diseases, such as HIV
47 infection (Douek et al., 1998) or the decline with age can be slowed down by diseases such
48 as acute myocardial infarction (Lakhonina et al., 2012). This technique has so far been used
49 to determine age in humans (Cho et al., 2014) and dogs (Ito et al., 2015). More information
50 on this technique can be found in a review by Jarman et al. (2015).

51 Racemization

52 In metabolically inactive tissues, such as teeth and eye lens nuclei, the L-enantiomer (L) of
53 aspartic acid converts to the D-enantiomer (D) over time, a process called racemization
54 (Garde et al., 2010). This racemization occurs at a relatively constant rate throughout the
55 life of an individual (Garde et al., 2010), although the process can be accelerated by high
56 body temperatures (Bada and Brown, 1980). It is possible to estimate the age of an animal
57 when its D/L ratio at birth and the racemization rate are known (Wehmille and Hare, 1971).
58 Racemization has been widely applied to determine age in humans using teeth (Ohtani and
59 Yamamoto, 2005) and has also occasionally been employed in marine mammals using eye
60 lenses (Garde et al., 2007, Garde et al., 2010) and birds using tendons (Hunter et al., 2003).
61 Although the application of racemization has thus mainly been in humans and much less
62 frequently in other taxa, we include this aging technique into our review to evaluate its
63 potential use to a wider range of taxa in the future.

64 Otolith ring count

65 Otoliths are hard structures embedded in the inner ears of bony fishes and are primarily
66 comprised of calcium carbonate (Degens et al., 1969). Growth rings in otoliths are suggested
67 to be formed annually in a large number of fish species (Gooley, 1992, Santana et al., 2009).

Otolith ring count has generally been perceived as a highly accurate aging method. However, growth rings may not be deposited every year (Fowler, 1995) and false (non-annual) rings can appear, e.g. due to critical moments in development, such as sex maturity (Khan and Khan, 2009). Nevertheless, otolith ring count has developed into a traditional aging technique in fishes, ages inferred from this technique frequently being assumed to represent "true" age, and subsequently used to validate other aging techniques, such as the below discussed otolithometry and age-length keys.

Otolithometry

Otoliths grow throughout life without reabsorption (Campana, 1999). The strong linear correlations between both otolith size (i.e. length, breadth and height) and weight with age (Steward et al., 2009) were confirmed in a number of species. Otolithometry therefore was proposed as another potential approach to age fish (Fossen et al., 2003).

Age-length keys

In addition to otolith ring count and otolithometry, age-length key, which is the correlation between body length and age (class), has also been widely applied in studies in fish. Von Bertalanffy's growth model is among the most popular models used to describe age-length keys: $L_t = L_\infty(1 - e^{-k(t-t_0)})$, where L_t is the observed mean length at age t, L_∞ is the maximum length fish can theoretically achieve, k is the growth constant and t_0 the theoretical age at zero length (Templeman and Squires, 1956). To ease fitting of growth data to this exponential age-length equation, it is commonly converted into a linear model:

$$\ln\left(1 - L_t/L_\infty\right) = kt_0 - kt.$$

Skeletochronology

Similar to growth rings in otoliths, growth rings in bones are formed by seasonal growth and are suggested to increment annually (de Buffrénil and Castanet, 2000, Snover et al., 2011, Friedl and Klump, 1997). The use of growth rings to reconstruct growth history and estimate age of an individual is termed skeletochronology. This technique has mainly been used in reptiles and amphibians, but also in mammals and birds (Sinsch, 2015). As for otolith ring counts, skeletochronology has been perceived as a highly accurate aging method and frequently assumed to represent true age (e.g. Cogălniceanu et al., 2017, Otero et al., 2017, Comas et al., 2016). However, age can be overestimated by noncyclic growth rings introduced by irregular environmental conditions, interrupting the seasonal growth pattern (e.g. elevated ambient temperature that interrupts hibernation (Sinsch et al., 2007)). Age could be underestimated if growth rings are damaged by factors such as bone reabsorption (Bjorndal et al., 1998) and rapprochement of (mostly peripheral) growth rings (Eden et al., 2007). More information on this technique can be found in a review by Sinsch (2015).

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